METHOD FOR PREPARING POLYSACCHARIDE OF GREEN TEA AND COSMETIC COMPOSITION FOR SKIN WHITENING, MOISTURIZATION AND ANTI-WRINKLE EFFECTS COMPRISING THE POLYSACCHARIDE

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None

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U.S. PATENT DOCUMENTS

ABSTRACT

Disclosed is a method for preparing polysaccharides of green tea, and more particularly to a method for preparing polysaccharides of green tea comprising the steps of: a) removing chlorophyll and a low molecular weight polyphenol from green tea powder using a solvent; b) hot-water extracting a water-soluble active ingredient from the green tea residue of step a); and c) separating the polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation. Also, the present invention relates to a cosmetic composition for skin whitening, moisturization and anti-wrinkle effects comprising the polysaccharides of green tea as an effective ingredient.

10 Claims, 2 Drawing Sheets
Figure 1

Figure 2
Figure 3

A bar graph showing the effect of UV irradiation and polysaccharides of green tea on a certain parameter. The x-axis represents different conditions: No UV irradiation, UV irradiation, RA 10 µM, 50 ppm, 10 ppm, and 1 ppm Polysaccharides of green tea. The y-axis represents the percentage of control, ranging from 0 to 160%. The graph indicates a significant increase in the parameter with UV irradiation and a decrease with the addition of polysaccharides.
METHOD FOR PREPARING POLYSACCHARIDE OF GREEN TEA AND COSMETIC COMPOSITION FOR SKIN WHITENING, MOISTURIZATION AND ANTI-WRINKLE EFFECTS COMPRISING THE POLYSACCHARIDE


TECHNICAL FIELD

The present invention relates to a method for preparing polysaccharides of green tea, and more particularly to a method for preparing polysaccharides of green tea comprising the steps of:

a) removing chlorophyll and low molecular weight polyphenols from green tea powder using a solvent;

b) hot-water extracting water-soluble active ingredients from the green tea residue of step a); and

c) separating the polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation. Also, the present invention relates to a cosmetic composition for skin whitening, moisturization and anti-wrinkle effects comprising the polysaccharides of green tea as an effective ingredient.

BACKGROUND ART

Green tea contains many active ingredients of benefit to humans. These active ingredients include catechins such as epicatechin, epigallocatechin, epicatechingallate and epigallocatechingallate, which are known for anti-tumor effect, anti-oxidizing effect, anti-bacterial effect, detoxication of heavy metals and suppression of high blood pressure.

Nowadays, studies are being actively conducted to search for pharmacological active substances in the green tea other than the catechins. Polyphenols extracted from the green tea are reported to have an inhibitory effect on photo-aging and can be used as a UV protecting agent. Also, gallocatechingallate as an isomer is studied for its extraction. Further, dermal formulations for external application comprising the green tea extract are studied for improvement of wrinkling and elasticity deterioration caused by skin aging. This is because the polyphenols contained in the green tea can eliminate free radicals and activate defense factors that are related to the anti-oxidation in the skin, and thereby, improve wrinkling and skin elasticity.

In addition, water soluble polysaccharides existing in the green tea are known to have immunization, anti-radioactivity, anti-coagulant, anti-tumor, anti-HIV and reduction of blood glucose level activities. The polysaccharides isolated and purified from the green tea have an ability to suppress the bonding between Propionibacterium acne and atopic Staphylococcus aureus and the bonding between Helicobacter pylori and host cells in the human body. In cited references, ion exchange and gel filtration chromatography are used to separate the polysaccharides of the green tea. However, the ion exchange and gel filtration chromatography processes are inferior in terms of economical efficiency since they require excessive time, and no techniques for mass production have yet been established.

DISCLOSURE

Technical Problem

Accordingly, the present inventors have conducted studies to seek a method for preparing polysaccharides of green tea in a more economical way, and as a result, have developed a method for preparing polysaccharides of green tea comprising the steps of:

a) removing chlorophyll and low molecular weight polyphenols from green tea powder using a solvent;

b) hot-water extracting water-soluble active ingredients from the green tea residue of step a); and

c) separating the polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation. By this method, it is possible to prepare the polysaccharides of green tea in a short process time and in large quantities, leading to an increase in economical efficiency. Based on this finding, the present invention has been completed.

Also, the inventors have found that a cosmetic composition comprising the polysaccharides of green tea shows excellent skin whitening, skin moisturizing and anti-wrinkle effects.

Therefore, an object of the present invention is to provide a method for preparing polysaccharides of green tea at a high economical efficiency.

Further, another object of the present invention is to provide a cosmetic composition for skin whitening, skin moisturizing and anti-wrinkle effects.

Technical Solution

To achieve the above object, the present invention provides a method for preparing polysaccharides of green tea comprising the steps of:

a) removing chlorophyll and low molecular weight polyphenols from green tea powder using a solvent;

b) hot-water extracting water-soluble active ingredients from the green tea residue of step a); and

c) separating the polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation.

In another aspect, the present invention provides a cosmetic composition for skin whitening, moisturizing and anti-wrinkle effects comprising the polysaccharides of green tea as an effective ingredient.

Advantageous Effects

When polysaccharides of green tea are prepared by the method according to the present invention, it is possible to
reduce the production time and to produce the polysaccharides of green tea in large quantities, leading to an improvement in economical efficiency. Also, the cosmetic composition comprising the polysaccharides of green tea as an effective ingredient according to the present invention can provide excellent skin whitening, moisturizing and anti-wrinkle effects without skin irritation.

DESCRIPTION OF DRAWINGS

FIG. 1 is a graph showing the result of a cytotoxicity test of the polysaccharides of green tea.

FIG. 2 is a graph showing the inhibitory effect of the polysaccharides of green tea on production of melanin.

FIG. 3 is a graph showing the inhibitory effect of the polysaccharides of green tea on development of matrix metalloprotease-1 (MMP-1).

BEST MODE

The present invention relates to a method for preparing polysaccharides of green tea, and more particularly to a method for preparing polysaccharides of green tea comprising the steps of:

a) removing chlorophyll and low molecular weight polyphenols from green tea powder using a solvent;

b) hot-water extracting water-soluble active ingredients from the green tea residue of step a); and

c) separating the polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation.

Also, the present invention relates to a cosmetic composition for skin whitening, moisturization and anti-wrinkle effects comprising the polysaccharides of green tea as an effective ingredient.

Therefore, the present invention is described in detail.

The polysaccharides of green tea according to the present invention are derived from *Camellia sinensis*, a member of tea trees belonging to the family Theaceae and the genus *Camellia*, which is an evergreen shrub known to have immunization, anti-radiocactivity, anti-coagulant, anti-tumor, anti-HIV and reduction of blood glucose level activities. Also, the polysaccharides of green tea suppress generation of melanin and expression of matrix metalloprotease-1 (MMP-1) and increase skin moisturization, without cytotoxicity, which leads to skin irritation, whereby it can show skin whitening, skin moisturizing and anti-wrinkle effects.

The individual steps of the method for preparing polysaccharides of green tea according to the present invention are described in detail below.

Step a) to remove chlorophyll and low molecular weight polyphenols from the green tea powder using a solvent:

In order to remove chlorophyll and low molecular weight polyphenols from the green tea powder, solvent extraction is performed. The extraction solvent is preferably at least one selected from the group consisting of hexane, ethanol and methanol. In order to prevent toxicity that may be caused by the residue upon application to cosmetics, ethanol is more preferably used.

The solvent is added to the green tea powder and thoroughly stirred at room temperature. The solvent in which chlorophyll and low molecular weight polyphenols are dissolved is then removed by centrifugation. The resulting residue is dried to obtain a product containing no chlorophyll and low molecular weight polyphenols.

Step b) to hot-water extract water-soluble active ingredients from the residue of step a):

In order to extract water-soluble active ingredients from the residue prepared in step a), hot-water extraction is performed. The hot-water extraction is performed preferably at a temperature of 30 to 40°C. This is because if the temperature exceeds 40°C, thermal denaturation can occur, while if the temperature is lower than 30°C, the polysaccharides are not sufficiently extracted. Also, the hot-water extraction is performed preferably for 6 to 8 hours. This is because if the time exceeds 8 hours, contamination by microorganisms can occur, while if the time is less than 6 hours, the polysaccharides are not sufficiently extracted. The extract obtained from the hot-water extraction is filtered using a filtering apparatus and concentrated in vacuo.

Step c) to separate polysaccharides of green tea from the hot-water extract of step b) by ultrafiltration and ethanol precipitation:

In order to separate polysaccharides from the hot-water extract of step b), ultrafiltration and ethanol precipitation is performed. The polysaccharides comprise about 60 to 65% of a polysaccharide part and about 8 to 9% of a protein part.

These two parts are connected by amino acid-sugar bond and the rest comprises protein isolates. Therefore, the polysaccharides have molecular weights of 100,000 to 300,000 daltons, which are much greater than those of the protein isolates. By means of this molecular weight difference, the ultrafiltration is performed to separate the polysaccharides of green tea. Also, the ultrafiltration has merits in that it is a continuous process capable of simultaneously performing separation of low molecular weight substances and concentration of the filtrate and in which thermal denaturation does not occur, since the process is practiced at room temperature. Then, ethanol is slowly added to the ultrafiltration concentrate to perform the ethanol precipitation. Here, ethanol is added preferably at a rate of 100 to 200 ml/min. If ethanol is added at a rate faster than 200 ml/min, the final product particles are too big and bulk up. After completion of the ethanol precipitation, ethanol is removed and the product is dried in vacuo at a temperature of 40 to 50°C to obtain the polysaccharides of green tea in the powder form.

The polysaccharides of green tea according to the present invention show excellent thermal stability, pH stability and body stability as well as formulation stability. Also, according to the present invention, it is possible to prepare the polysaccharides of green tea at a relatively high yield.

Since the polysaccharides of green tea according to the present invention do not contain ingredients such as polyphenols, which are a main cause of discoloration, unlike the conventional green tea extract, which is limited in its use due to the problems of discoloration, they can be used in a large amount in formulations. Therefore, the cosmetic composition comprising the polysaccharides of green tea can have maximized skin whitening, moisturizing and anti-wrinkle effects without additional effective ingredients.

Therefore, the cosmetic composition according to the present invention comprises the polysaccharides of green tea as an effective ingredient and shows excellent skin whitening, moisturizing and anti-wrinkle effects. The cosmetic composition is not particularly limited in its formulation and can be formulated into skin softener, lotion, moisturizing lotion, massage cream, pack, gel, body lotion, body oil and body essence. Each formulation of the cosmetic composition may include other ingredients than the polysaccharides of green tea according to the formulation type and final use, and the additional ingredients can be properly selected by those skilled in the art without difficulty.

Further, the cosmetic composition comprises the polysaccharides of green tea preferably in an amount of 0.1 to 20%
by weight based on the total weight of the composition. If
the polysaccharides of green tea are contained in an amount
of more than 20% by weight, the formulation stabilization
such as phase separation can be affected. If the polysaccha-
rides of green tea are contained in an amount of less than
0.1% by weight, the effects of the formulation are limited.

Hereinafter, the present invention is described in further
detail with reference to examples. It should be understood,
however, that these examples are for illustrative purposes
only and are not to be construed to limit the scope of the
present invention.

EXAMPLES

Example 1

Preparation of Polysaccharide of Green Tea

Dried green tea leaves were pulverized and sieved
through a screen to obtain green tea powder (d50=100–1000
μm). 10 kg of the green tea powder was dispersed in 150 l
of 95% (v/v) ethanol and stirred at room temperature.
The mixture was centrifuged to remove chlorophyll and low
molecular weight polyphenols. The residue was extracted
once with the same solvent and dried.

The product of the extraction, from which the chlorophyll
and low molecular weight polyphenols had been removed,
was added to 125 l of water and stirred at 35°C for 7 hours
for hot-water extraction. The resulting extract of the poly-
saccharide of green tea was filtered through a filter press,
collected and concentrated in vacuo at 62°C to 3/5 of the
initial volume.

The concentrate of the polysaccharides of green tea was
ultrafiltered (molecular weight CUT OFF: 30,000 daltons) to
remove low molecular weight protein isolates, and then
subjected to the ethanol precipitation by slowly adding
ethanol in an amount of 5 times of the final ultrafiltrate at
a rate of 100 ml/min. The precipitated polysaccharides of
green tea were dried in vacuo at 45°C to obtain 250 g of
the polysaccharides of green tea as powder.

Experimental Example 1

Cytotoxicity Test

Human keratinocyte HaCaT cells were seeded in a
96-well plate, each well containing 1×10^4 cells, and cultured
for 24 hours. The plate was washed once with 100 μl of PBS
(phosphate buffered saline), exchanged with fresh medium
containing 1, 10, 50 and 100 ppm of the polysaccharides of
green tea, and cultured for 48 hours. WST-1 solution (Roche
Diagnostic GmbH, Germany), diluted 10 times, was added
to each well. The plate was incubated at 37°C for 2 hours.
Subsequently, the absorbance at 450 nm of the resulting
solution was measured. A control group used dimethyl
sulfoxide to dissolve the sample. The data of the adsorption
were corrected for the data of the control group and the
result is shown in FIG. 1.

As shown in FIG. 1, it was noted that the samples
containing the polysaccharides of green tea according to the
present invention showed cell viabilities similar to the
control group and did not have cytotoxicity or induce skin
irritation in the used concentration range.

Experimental Example 2

Inhibitory Effect on Melanin Formation

In order to compare the inhibitory effect of the acidic
polysaccharides of green tea on melanin formation in cells,
this example was performed using arbutin, which is known
as a melanin biosynthesis inhibitor, as a comparator. Melanin
A cells were seeded into a 24-well plate, each well
containing 3×10^4 cells. One day later, the wells of the plate
were treated three times with either 10, 50 and 100 ppm of
arbutin or with 1, 10, 50 and 100 ppm of the polysaccharides
of green tea. After incubation at 37°C for 3 days, the wells
of the plate were again treated with arbutin or the polysaccha-
rides of green tea by the same method as described
above. After incubation for a further 3 days, the plate was
washed with PBS, 1N NaOH solution was used to dissolve
the formed melanin, and subsequently the absorption at 405
nm of the resulting solution was measured. The control
group used dimethyl sulfoxide to dissolve the sample. The
data of the absorption were corrected for the data of the
control group and the result is shown in FIG. 2.

As shown in FIG. 2, it was noted that the samples
containing the polysaccharides of green tea according to the
present invention inhibited the melanin synthesis in propor-
tion to the concentration and that the polysaccharides of
green tea have an IC50 of 50 ppm. Therefore, the polysaccha-
rides of green tea according to the present invention showed
an inhibitory effect on melanin synthesis similar to that of arbutin. The IC50 was determined as a concentration
at which the absorption was reduced to 50%.

Experimental Example 3

Inhibitory Effect on Expression of MMP-1 by UV

In order to confirm the inhibitory effect on expression of
matrix MMP-1 increased by exposure to UV, fibroblasts (p6)
were seeded in a 12-well plate, each well containing 0.75×
10^5 cells, and starved in serum-free medium for 24 hours.
The starved cells were washed with PBS and irradiated by
UV (40 mJ). Then, the cells were injected twice with either
10 μM retinoic acid (RA), or with 1, 10 and 50 ppm of the
polysaccharides of green tea during a 48 hours period.
The isolated MMP-1 in the medium was measured using a kit
(Amersham, RPN2610). The data were corrected for the
data of a non-UV irradiated control group and the result is
shown in FIG. 3.

As shown in FIG. 3, it was noted that the samples
containing the polysaccharides of green tea according to the
present invention inhibited the expression of MMP-1 in
proportion to the concentration. Particularly, at the concen-
tration of 50 ppm, the polysaccharides of green tea inhibited
the expression of MMP-1 at a level similar to the control
group that was not irradiated by UV.

Formulation Example 1 and Comparative Example
1: Preparation of Lotion

Formulation Example 1 and Comparative Example 1 were
prepared according to the composition described in Table 1
by following the conventional method (unit % by weight).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Example 1</th>
<th>Comparative Example 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified water</td>
<td>To 100</td>
<td>To 100</td>
</tr>
<tr>
<td>Polysaccharides of green tea of Example 1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Bees wax</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Polysorbote 60</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Sorbitan sesquioleate</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
TABLE 1-continued

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Example 1</th>
<th>Comparative Example 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana 202 (Seppic)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycerin</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Butylene glycol</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Carboxyvinyl polymer</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Triethanol amine</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Experimental Example 4

Increase of Skin Moisturizing Effect on Human Skin

50 male and female adults, 50 to 60 years old, with Xerosis cutis were divided into two groups and given the lotions prepared in Formula Example 1 or Comparative Example 1. The lotion was applied on the face twice a day for 4 weeks. Before the application, the skin's moisture level was measured as a reference using a corneometer in a controlled condition of a constant temperature of 24°C and a relative humidity of 40%. 1, 2 and 4 weeks after the application was initiated, and 2 weeks after the application was suspended, the skin's moisture level was measured. The result is shown in Table 2. The data show the increase of the skin's moisture level after the application for the predetermined period of time on the basis of the value measured by the corneometer before the experiment.

TABLE 2

<table>
<thead>
<tr>
<th>Test material</th>
<th>After 1 week</th>
<th>After 2 weeks</th>
<th>After 4 weeks</th>
<th>2 weeks after suspension of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>32</td>
<td>42</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>Example 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparative</td>
<td>29</td>
<td>33</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>Example 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As shown in Table 2, Formulation Example 1 containing the polysaccharides of green tea according to the present invention showed a greater increase rate than Comparative Example 1 not containing the polysaccharides of green tea. Further, even 2 weeks after the application was suspended (total 6 weeks passed), Formulation Example 1 maintained the skin's moisture level similar to those at 1 to 2 weeks after the application was initiated, which indicates that Formulation Example 1 can maintain the skin's moisture level for a certain period of time even after the application is suspended.

The invention claimed is:

1. A cosmetic composition comprising the polysaccharides of green tea as an effective ingredient, wherein the polysaccharides of green tea are characterized in that having a molecular weight of 100,000 to 300,000 daltons, with no having low molecular weight free proteins of below 30,000 Daltons and chlorophyll, wherein the polysaccharides of green tea are prepared by the method comprising the following steps of:
   a) removing chlorophyll and low molecular weight polyphenols from green tea powder using a solvent;
   b) hot-water extracting water-soluble active ingredients from the green tea residue of step a) at a temperature of 30 to 40°C for 6 to 8 hours; and
   c) separating the polysaccharides of green tea removing low molecular weight free proteins from the hot-water extract of step b) by ultrafiltration and then separating the polysaccharides of green tea by ethanol precipitation wherein the polysaccharides of green tea have a molecular weight of 100,000 to 300,000 daltons.

2. The cosmetic of claim 1, wherein the solvent used in step a) is at least one selected from the group consisting of hexane, ethanol and methanol.

3. The composition of claim 1, wherein the molecular weight cut-off of the ultrafiltration is 30,000 Daltons.

4. The composition of claim 1, wherein the polysaccharides of green tea are contained in an amount of 0.1 to 20% by weight based on the total weight of the composition.

5. The composition of claim 1, wherein the cosmetic composition is used for skin whitening.

6. The composition of claim 1, wherein the cosmetic composition is used for skin whitening.

7. The composition of claim 1, wherein the cosmetic composition is used for skin moisturization.

8. The composition of claim 1, wherein the cosmetic composition is used for skin moisturization.

9. The composition of claim 1, wherein the cosmetic composition is used for improving skin wrinkle.

10. The composition of claim 1, wherein the cosmetic composition is used for improving skin wrinkle.

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